Evaluation of CD98 Expression in Normal and Osteoarthritic Human Articular Chondrocytes

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ABSTRACT

Background: Recent studies have provided evidence that integrins play roles in recognition of mechanical stimuli and its translation into a cellular response. Integrin signaling may be regulated by a number of mechanisms including accessory proteins such as CD98 (4F2 antigen). **Objectives:** To determine CD98 expression by human articular chondrocytes and its involvement in human articular mechanotransduction. **Methods:** CD98 expression was assessed by immunostaining of cryostat sections of snap frozen articular cartilage and in cultured cells by western blotting. Chondrocytes enzymatically isolated from macroscopically normal and osteoarthritic (OA) articular cartilage were grown in short term, primary monolayer culture and used in a resting state or following mechanical stimulation at 0.33Hz. **Results:** Human articular chondrocytes express CD98 and immunoreactivity revealed a similar heterogeneous pattern of CD98 in both normal and osteoarthritic (OA) human articular cartilage. No role of CD98 was detected by electrophysiological study. **Conclusion:** It appears that CD98 is expressed in a similar pattern in both normal and osteoarthritic (OA) cartilage. Although we detected no role for CD98 in chondrocyte mechanotransduction, it may be involved in other biological functions in chondrocyte intracellular signalling events.

Keywords: CD98, 4f2 Antigen, Articular Cartilage, Integrin, Mechanotransduction

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INTRODUCTION

Articular cartilage is subjected, in vivo, to frequent and repetitive mechanical loads which regulate chondrocyte function. Previous studies have demonstrated that human articular chondrocytes show changes in membrane potential following cyclical mechanical stimulation. In normal chondrocytes, a membrane hyperpolarisation response to mechanical stimulation at 0.33 Hz is mediated by $\alpha5\beta_1$ integrin and is associated with interleukin 4. In contrast, osteoarthritic (OA) chondrocytes show a membrane depolarization to the same stimulus. This involves $\alpha5\beta_1$ integrin and tyrosine kinase activity but is not influenced by cytochalasin D, which disrupts the actin cytoskeleton and staurosporine (an inhibitor of PKC) (1). $\alpha5\beta_1$ integrin appears to be the mechanoreceptor in both cell types but activated down stream pathways appear to be different. The reasons for these differences in mechanotransduction between normal and OA human articular chondrocytes are unclear. Integrin function has been shown to be modified by accessory molecules including CD98. Altered expression and interaction of such integrin associated molecules in osteoarthritis would be expected to modify chondrocyte function and such interactions may be important in response to mechanical stimulation or other integrin dependent cell matrix interactions regulating chondrocyte behavior.

CD98 is a widely expressed 120 kD heterodimeric cell surface protein, originally identified as the 4F2 antigen associated with T cell activation (2). It is composed of an 85 kD heavy chain and a 40 kD light chain. A variety of cell functions have been shown to involve CD98 including cell fusion and cell aggregation (3). CD98 physically and functionally interacts with integrin receptors in multiple cell types (4) and studies have shown that CD98 modulates the activity of β_1 integrins (5).

We undertook this study to evaluate the expression and function of CD98 in normal and OA human articular cartilage mechanotransduction.

MATERIALS AND METHODS

Source of Tissue and Chondrocyte Culture. Human articular cartilage was obtained, with ethical approval and patients' consent, from knee joint arthroplasty specimens and amputations following peripheral vascular disease. Cartilage was assessed macroscopically for the presence or absence of OA changes and was graded for osteoarthritis using the Collins/McElligott (6) system. Chondrocytes were isolated by sequential enzyme digestion and cells were seeded in Iscove's modified Dulbecco's medium (Gibco) supplemented with 10 % fetal calf serum (Sigma), 100 I.U./ml penicillin (Gibco), and 100 µg/ml streptomycin (Gibco) to a final density of $5x10^5$/ml. Primary non-confluent 1-2 weeks old cultures of chondrocytes were used in all experiments. The day before mechanical stimulation, culture media containing serum was replaced by serum-free media.

Immunohistochemistry. Samples of articular cartilage were snap frozen in liquid nitrogen for cryostat sectioning or fixed in 4% formaldehyde for production of paraffin embedded sections. Immunoreactivity was assessed by the relative number of posi-

tive and negative chondrocytes in different zones of cartilage (7). Evaluation was carried out three times for each section.

For CD98, immunostaining was performed on cryostat sections obtained from 11 knee joints, 6 men and 4 women (age range 39-85, mean 67 years), and 9 femoral heads including 4 men and 5 women (age range 73-90, mean 81 years) using an avidin-biotin conjugated (ABC) immunoperoxidase method with the mouse monoclonal antibody 4F2 (Serotec).

Induction of Cyclical Mechanical Stimulation. The technique and apparatus used for mechanical stimulation of primary human articular chondrocytes have been previously described in details (8). 55 mm diameter plastic tissue culture dishes (Nunc) containing sparse primary monolayer cultures of human articular chondrocyte monolayer were placed in a sealed pressure chamber with inlet and outlet ports. The chamber was pressurised using helium gas from a cylinder, at a determined frequency by an electronic timer that controlled the inlet and outlet valves. The standard stimulation regimen used was a frequency of 0.33 Hz (2 sec on, 1 sec off) for 20 min at 37°C and a pressure of 16 kPa above atmospheric pressure. This system produces 3700 microstrains on the base of the culture dish.

Protein Extraction and Western Blotting. Methods used for protein extraction, immunoprecipitation, and western blotting have been described previously (7).Cells at rest or following mechanical stimulation were washed with ice-cold PBS containing 100 μM Na_3VO_4 (Sigma) and lysed in situ with ice-cold lysis buffer containing 1% Igepal (Sigma), 100 μM Na_3VO_4, and protease inhibitor cocktail tablet (Boehringer Mannheim) at 4°C for 15 min. Supernatants were collected after centrifugation at 13000 rpm for 15 min. Whole cell extracts were separated on SDS-PAGE under reducing conditions. Following electrophoresis, cell lysates were transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore Immobilon-P, Sigma). Membranes were blocked overnight at 4°C with 2% BSA in TBST (12.5 mM Tris/HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20). After washing with TBST, blots were incubated for 1 h at room temperature with primary antibodies and then HRP labelled secondary antibodies. Membranes were rewashed extensively and bands were detected using Enhanced Chemiluminescense Plus (Amersham).

Blots were incubated with polyclonal goat anti-CD98 (IgG) (Santa Crus) at 1: 500 as primary antibody. At least 3 samples from different donors for normal and OA cartilage were analysed.

Recording of Membrane Potential. Membrane potentials of cells were recorded using a single electrode bridge circuit and calibrator (8). Membrane potentials of isolated cells were measured, and results were accepted if, on cell impalement, there was a rapid change in voltage to the membrane potential level that remained constant for at least 60 s. The membrane potentials of 5-10 cells were measured prior to and following addition of the reagent to be tested and/or mechanical stimulation. Each experiment was undertaken at least three times on cells from different donors. The following antibodies were used: polyclonal goat anti-CD98 (SantaCrus) at 1.5μg/ml; mouse anti-CD98 monoclonal antibodies BU89 and BU53 (kindly provided by Dr Hardie) at 1:100. Non-immune serum was used for negative controls.

Statistical Analysis. The mean and standard error of mean of cell membrane potentials were determined in each experiment. For statistical comparisons, when the F-ratio of the two variances reached significance, non-parametric Mann-Whitney test was used. When the ratio did not reach significance, Student t-test was used.

RESULTS

Expression of CD98 in Human Articular Chondrocytes (Immunohistochemistry). Sixteen sections of normal articular cartilage were obtained from 4 men (age range 39-73, mean 55 years) and 7 women (age range 67-83, mean 75 years). Normal articular cartilage sections were isolated from 5 femoral heads (all female, age range 73-90, mean 79 years), 6 femoral condyles (3 females, age and sex of one case was not recorded, age range 39-74, mean 64 years), 6 tibial plateaux (3 females, age and sex of one case was not recorded, age range 44-73, mean 63 years), and one patella (male, age 39).
Chondrocytes in all zones of normal articular cartilage revealed positive immunoreactivity with a similar expression pattern. The median scores (ranges) for surface, superficial, mid, and deep zones were 4 (2-5), 4 (3-5), 4 (2-4), and 4(2-4), respectively. There was no statistical significant difference in immunoreactivity of chondrocytes in surface and deep zones (Figure1, Table1). Immunoreactivity was characterized by a heterogeneous staining pattern. Some cells were positive, others negative. The age and gender of cases had no significant effect on the pattern of CD98 expression.

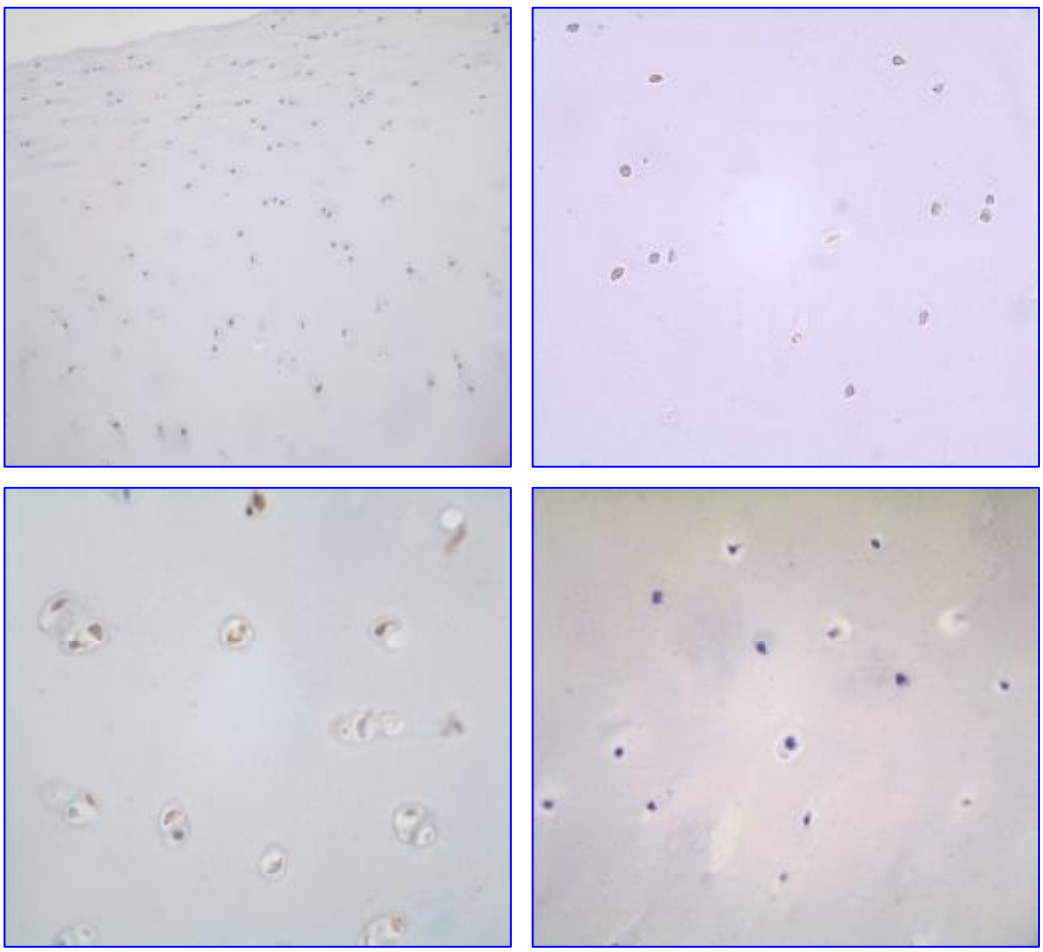

Figure 1. *Immunoreactivity of mAb anti-CD98 4F2 in normal adult human articular cartilage sections. CD98 positive and negative chondrocytes were assessed in all zones of normal*

articular cartilage using 4F2 mAb at 1:50. A: Femoral head (superficial-deep zone) of a 74-year-old female (x100). In all zones from surface to deep, chondrocytes show a heterogeneous pattern of CD98 expression. B: Tibial plateaux (mid zone) of a 72-year-old female (x200). Chondrocytes in mid zone show a heterogeneous pattern similar to other zones. C: Femoral condoyle (mid zone) of a 74-year-old female (x400). D: Negative control, femoral head (mid zone) of an 83-year-old female (x200). No positive signal was observed in F/H section of negative control.

Table 1. Immunoreactivity of CD98 in normal and OA articular cartilage sections isolated from hip (femoral head) and knee (tibia, femoral condyle, and patella) joints. In normal and mild OA surface zones compared with all zones P-value was >0.05. In severe OA clones grade compared with the other zones P-value was >0.05.

Sample	Immunostaining Score(Mean ± SEM)					
	n	Surface	Superficial	Clones	Middle	Deep
Normal	16	3.8 ± 0.2	4±0.1	__	3.5±0.1	3.5±0.1
Mild OA	17	4.2 ± 0.1	3.8±0.1	__	3.5±0.12	3.5±0.2
Severe OA	7	N/A	N/A	5	4.1±0.3	4±0.2

N/A: Not available

CD98 expression was assessed in 17 mild (3 grade I, 14 grade II) and 7 severe (6 grade III, 1 grade IV) OA articular cartilage sections (7). Samples of OA articular cartilage were obtained from 8 males (age range 62-85, mean 74 years), 9 females (age range 67-90, mean 75 years), and one case whose age and sex was not recorded. Sections of mild OA articular cartilage were obtained from 5 femoral heads(4 females, age range 73-90, mean 79 years), 4 femoral condyles (2 females, 1 case whose age and sex was not recorded, age range 67-74, mean 71 years), 6 tibial plateau (3 females, one case whose age and sex was not recorded, age range 67-85, mean 73 years), and 3 patellae (all male, age range 64-74, mean 67 years) . Samples of severe OA articular cartilage were obtained from 4 femoral condyles (all male, age range 62-82, mean 72 years), 1 tibial plateaux (62-year-old male), and 2 patellae (male, 73 and 82-year-old).

Articular cartilage from mild OA exhibited a heterogeneous staining pattern in all zones of different joints. The median scores (range) for surface, superficial, mid, and deep zones were 4 (3-5), 4 (3-5), 4 (2-5), and 4 (2-5), respectively. In severe OA sections, chondrocytes in clusters and in areas of surface fibrillation showed a heterogeneous expression pattern similar to other zones. The median scores (range) for clones, mid, and deep zones were: 5, 4(3-5), and 4(3-5), respectively. There was no detectable difference in the pattern of CD98 expression between normal and different grades of OA (Table 1, Figure 2). The age and sex of donors did not have significant effect on the pattern of CD98 expression. There was variable expression of CD98 by human articular chondrocytes in both normal and OA cartilage.

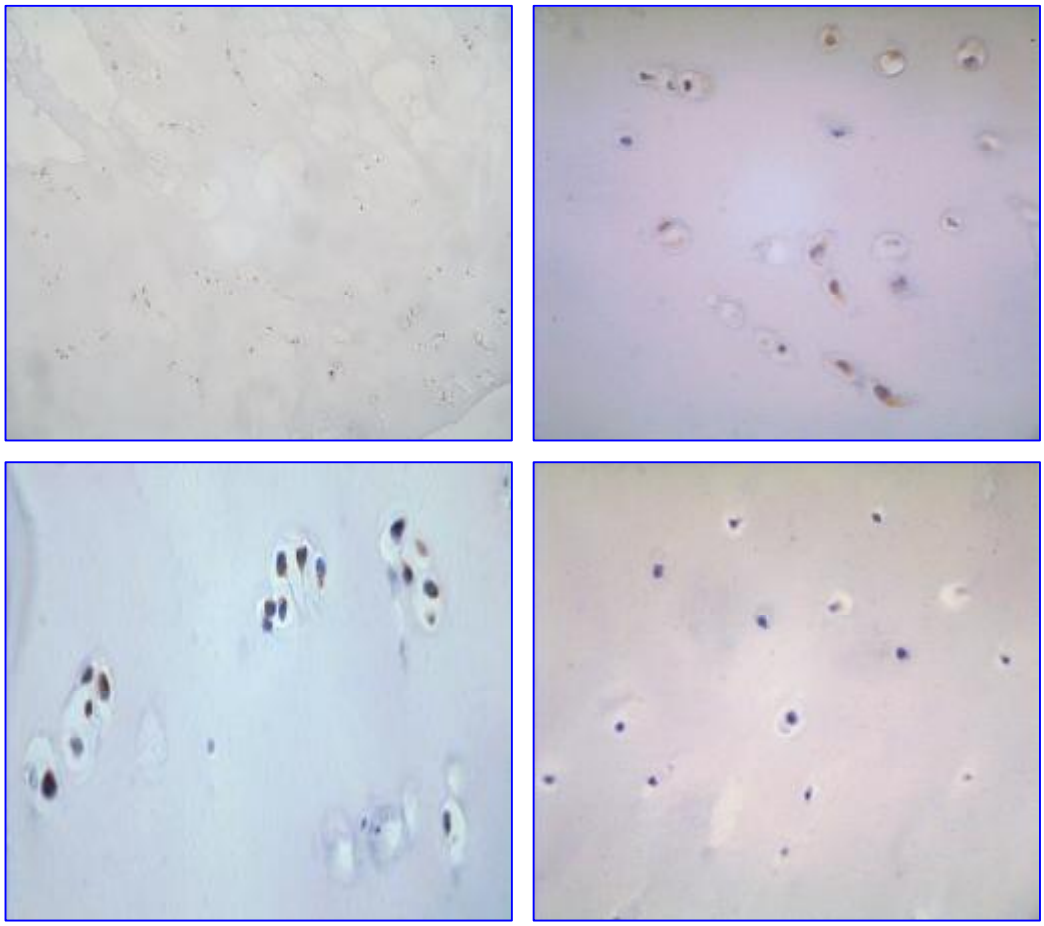

Figure 2. *Immunoreactivity of mAb anti-CD98 4F2 in OA human articular cartilage sections. CD98 positive and negative chondrocytes were assessed in all different zones of OA articular cartilage using 4F2 mAb at 1:50. A: Femoral condyle (surface-deep zone, OA III) of a 62-year-old male, (x100). From clones toward deep zone of OA femoral head, all chondrocytes show a similar heterogeneous expression pattern for CD98. B: Tibial plateaux (mid-zone, OA II) of a 67-year-old female, (x200). Chondrocyte in mid-zone show a similar heterogeneous pattern of CD98 C: Femoral head (surface-mid zone, OA II) of a 74-year-old female, (x400). High magnification of a clone from FAII showed heterogeneous expression of CD98. D: Negative control, femoral head (mid-zone) of an 83-year-old female, (x200). No positive signal at all was observed in F/H section of negative control.*

Western Blotting Analysis. Normal chondrocytes were isolated from 1 tibial plateaux (72-year-old male), 3 femoral heads (one female, age range 70-83, mean 78 years). OA chondrocytes were isolated from 5 femoral condyles (1 female, age range 69-87, mean 76 years,1 grade I, 1 grade II, and 3 grade III), 2 tibial plateaux (both female, 68 and 74-year-old, both grade II).

Cell lysates obtained from cultured chondrocytes and isolated from normal and OA cartilage were analyzed under reducing conditions and probed with goat polyclonal anti-CD98 at 1:500. As shown in figure 3, a similar single band at ~85 kD was expressed by normal and OA chondrocytes. The intensity of the CD98 band in normal and OA extracts from donors of different age and sex were similar.

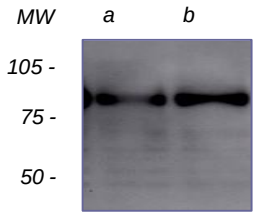

Figure 3. *CD98 in normal and OA human articular cartilage. Equal amounts (40μg/lane) of total cellular proteins prepared from the cultured articular chondrocytes derived from normal femoral head (lane a) and OA knee (lane b) were run in a 10% SDS-PAGE under reducing conditions. Heavy chain of CD98 expressed in chondrocytes were isolated from normal femoral head (lane a, male, age 70), and OA (lane b, tibial plateaux, female, age 87) cartilage under reducing conditions. Blots are representative of a series of experiments including at least three different donors (normal and OA). Molecular weights (MW) in kilo Daltons (kD) are indicated on the left.*

Roles of CD98 in Chondrocyte Mechanotransduction. Normal and OA chondrocytes were mechanically stimulated in the absence or presence of a panel of antibodies to CD98 (polyclonal goat anti-CD98, mouse monoclonal antibodies, BU89, and BU53). As shown in table 2, none of the anti-CD98 antibodies had effects on the resting membrane potential of chondrocytes, the hyperpolarisation response seen in normal chondrocytes, or the depolarization response seen in OA chondrocytes were obtained by following 0.33Hz mechanical stimulation.

Table 2. Effect of polyclonal anti-CD98 antibody on the hyperpolarisation response of normal cultured chondrocytes to 0.33 Hz cyclical mechanical stimulation

		Membrane potential (-mV) (Mean ± SEM)				
Sample	**Reagent**	**Resting**	**Anti-CD98**	**Anti-CD98&0.33 Hz MS**	**% Change**	**P-value**
	Nil	23.6±0.92	-	33.6±1.6	+42	0.0001
Normal	Anti-CD98	23.4±1.5	23.6±1.3	34±1.3	+45	0.0001
	Nil	33±1.4	-	23±1.5	-30	0.005
OA	Anti-CD98	33.2±1.5	31.6 ±1.5	24±1.7	-27	0.002
	Nil	30.6±2.2	-	20±1.5	-35	0.001
OA	BU89	35±1.9	31.2±3	24.8±1.5	-29	0.02
OA	BU53	26.8±1.2	25.8±1.4	18.6±0.01	-30	0.001

DISCUSSION

Several previous studies have shown that CD98 is implicated in multiple cell types and this supports the idea that this molecule probably plays a key role in cell signaling. The functional (4,5) and physical (9) association between CD98 and CD29 (β1 integrin) in the cell membrane are well documented and have led to the suggestion that CD98 signalling into the cell is in fact mediated via integrin activation. Several lines of evidence suggest that CD98 may modulate the integrin-mediated functions such as T cell co-stimulation (5), integrin-dependent adhesion (4,10), cell fusion (11), cell-cell fusion and aggregation (12), and amino acid transport (13).

It has been suggested that α5β1 integrin acts as a mechanoreceptor in human articular cartilage (1). Since integrins such as α5β1 have no intrinsic enzymatic activity of their own and also are not phosphorylated, they must interact with other proteins to generate signals (14).

In order to find a cell surface accessory molecule(s) for α5β1 integrin, we have focused on the detection and comparison in vivo and in vitro expression of CD98 in normal and OA human articular cartilage. It was noted that alterations in the expression or activity of one or more of the molecules involved in the signal transduction pathway could conceivably modify the response of diseased chondrocytes.

There is a possibility that due to CD98 interaction with ECM ligands, epitopes recognized by 4F2 antibody are masked. To find this out ,we can treat sections with proteases such as chondroitinase, which will remove ECM molecules, use a panel of antibodies against epitopes, or look at expression pattern of subpopulations of chondrocytes. Cho et al (15) have reported that CD98 antibodies are highly heterogeneous both in function and in the ability to bind to CD98 on the U937 cell surface. They have also demonstrated that it seems unlikely that this heterogeneity simply reflects variation in concentration or affinity of the antibodies, since each antibody was tested over a wide range of concentrations.

Consistent with in vivo immunohistochemical results, CD98 is expressed by normal and OA cultured articular chondrocytes. To obtain details of the exact state of CD98 expression in normal and different grades of OA chondrocytes, a number of samples in normal and OA cartilage are required.

In addition, normal and OA cultured chondrocytes treated with a panel of antibodies against CD98 revealed that these antibodies have no effect on electrophysiological response of chondrocytes to 0.33 Hz cyclical mechanical stimulation.

Using changes in the membrane potential as an indicator of chondrocyte response to mechanical stimulation, our initial investigations demonstrated that CD98 had no identifiable role in chondrocyte mechanotransduction. It might be reasonable to test the idea that CD98 forms a physical or functional complex on the chondrocyte cell membrane that potentially coordinates and regulates integrin-dependent functions and signaling in articular cartilage. However, it is equally possible that CD98 has a dynamic role in chondrocytes metabolic activity such as matrix receptors activity in cell-matrix interactions and protein transport.

To conclude, further studies are required to investigate the potential association of CD98 with chondrocyte integrins including α5β1 and involvement of CD98 in critical intracellular signaling pathways such as tyrosine phosphorylation. Results from this study do not exclude that CD98 might be involved in other biological functions and/or intracellular signaling events of chondrocytes.

ACKNOWLEDGMENT

This work was financially supported by a grant from Ahwaz Jondishapour University of Medical Sciences.

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